

**A REVIEW ON BIOMATERIAL DESIGN FOR VASCULAR TISSUE ENGINEERING
APPLICATIONS**

Siddharth Agarwal*, Aakash Parikh and Kirtesh Raut

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai,
India

*Corresponding author mail: siddharthagarwal92@gmail.com Telephone: +91 9987925738

ABSTRACT: Biomaterials play a critical role in the success of tissue engineering approaches as they guide the shape and structure of developing tissues, provide mechanical stability, and present opportunities to deliver inductive molecules to transplanted or migrating cells. Therefore, the selection of the appropriate biomaterial can have a profound impact on the quality of newly formed tissue. A major challenge facing the field of tissue engineering is the development or identification of materials capable of promoting the desired cellular and tissue behaviour. Given that few biomaterials possess all the necessary characteristics to perform ideally, engineers and clinicians alike have pursued the development of hybrid or composite biomaterials to synergize the beneficial properties of multiple materials into a superior matrix. The combination of natural and synthetic polymers with various other materials has demonstrated the ability to enhance various properties required for tissue engineering. In this review, we have made an attempt to review artificial components such as polymers, and strategies to design a composite which offer the desired physical properties. We also discuss synthesis methods for designer biomaterials and its applications in vascular tissue engineering. The continued development and implementation of hybrid biomaterials will lead to further successes in tissue engineering and regenerative medicine. The articles written with this goal in mind are by no means exhaustive and are only intended to stimulate the community to think more broadly about biomaterial composites and their designing.

Key words: Biomaterials, Tissue engineering, Composite, Regenerative medicine

INTRODUCTION

In biochemistry, the term biomaterials is used to refer to the three conventional biopolymer materials that are composed of proteins/polypeptides, carbohydrates, lipids, or nucleic acids, as well as non-polymeric molecules with large molecular mass such as lipids and macrocycles. As biomaterials scientists, we can draw inspiration from the naturally evolved structure–function relationships of biomolecules and then translate these relationships into designed biomaterials with specific properties. Design is the art of conceiving of and producing a plan of something before it is made. For this we address the need by creatively putting the information we have thereby making the material as per requirement. Biomaterial design consists of applying the foundations of biomaterials creatively to meet the requirement (Barker and Heilshorn 2014).

The term ‘composite’ is taken in its common form as meaning a structure consisting of two or more distinct parts. This definition is not applied to the molecular level and thus homogenous scaffolds comprised only of co-polymers are not considered within this review. This review presents examples of tissue engineered composites applicable to vascular systems (Jenkins, Kratochvíl et al. 1996). Tissue engineered therapies are necessary due to the lack of clinical treatments capable of restoring full functionality once a defect has occurred. One strategy to promote the regeneration of healthy tissue involves the implantation of material-cell hybrid constructs into lesions incapable of self-repair. Although a few tissue engineered products have managed to translate to practicing medicine, most have stalled in the laboratory as a result of unsuitable mechanical, biological, and fabrication properties (Davis and Leach 2008). Many researchers have tried to resolve these challenges by seeking out new biomaterials, cell sources, or inductive factors to increase appropriate regrowth for the replacement of diseased or damaged tissues. One particular strategy combines previously characterized biomaterials to create composites possessing beneficial attributes not present in its constituent components. This strategy has been attracting much attention lately since it appears promising as composite biomaterials with desired properties can be made in this fashion, which are very difficult to find in materials made entirely of one type of co-polymers (Davis and Leach 2008).

The first tissue-engineered blood vessel substitute was created by Weinberg and Bell in 1986. They generated cultures of bovine endothelial cells; smooth muscle cells (SMCs) and fibroblasts in layers of collagen gel supported by a mesh made of Dacron. Although physiologic pressures were sustained for about 3–6 weeks, hence the feasibility of a tissue engineered graft with human cells was demonstrated. Since then, strategies to create an appropriate material for avascular graft have focused on three major areas of research: coatings and surface chemical modifications of biopolymers, biodegradable scaffolds and synthetic materials (Weinberg and Bell 1986).

The central question that has fascinated biomedical researchers from the beginning has therefore been how to design and control material properties to achieve a specific biological response.

Here in this review, we have attempted to cover biomaterial design in tissue-engineering strategies for in situ vascular regeneration, in which the body's natural healing response is modulated by material design and fabrication, or strategies for ex vivo formation of a blood vessel substitute, whereby in vitro culture of human cells on polymer substrates before implantation defines their mechanical and biological properties.

METHODS

To approach the subject in a systematic way, we have reviewed each factor affecting the properties of the biomaterials separately. An attempt has been made into understanding these factors and to arrive at a solution to problems arising specifically due to that factor. Thus appropriate suggestions according to the requirement can be chosen from each segment and by combining them, a custom made composite as per the requirement can be made.

1. Design to reduce thrombogenicity

Thrombogenicity is a major concern while designing a biocomposite intended for in-vivo applications. Small vessels (< 6mm) in particular pose a worry for thrombogenicity since blood flow velocities are lower leading to increased potential of activating the coagulation cascade. To reduce thrombus formation, we can provide a coating of an appropriate anti-thrombotic agent on the surface of the biopolymer (Sakiyama-Elbert 2014).

To tackle the problem of thrombogenicity, the use of heparin and heparin sulphate has been well documented. They are linear polysaccharides, which are synthesized from a common precursor proteoglycan (more specifically testican). Heparin is only produced in mast cells, where it is cleaved from the core protein (serglycin) at the end of synthesis. Heparin sulfate (HS) is found in most tissues and remains attached to the core protein. Both are sulphated and also contain carboxylic acids, which contribute to an overall net negative charge. Heparin/HS polymer chains are made up of repeating disaccharides, primarily uronic acid and glucosamine with varying degrees of sulphation and N-acetylation (Iozzo 2001). Approach for attaching Heparin took advantage of electrostatic interactions of the negatively charged sulphate groups on Heparin with the biomaterial surface. Methods to attach them on biomaterial surfaces can be:

- Ionic interactions immobilization: Modifying surfaces by forming quaternary ammonium sites on the material surface to promote electrostatic interactions with heparin.
- End-point immobilization: covalent conjugation were developed that used end-point immobilization, in which a primary amine on the material of interest was reacted with an aldehyde group generated by heparin chain depolymerisation.

But now, newer polymers have been synthesized (Kannan, Salacinski et al. 2005) which have greater anti-thrombotic action and also display a great degree of compliance match to natural vascular tissues (Devine and McCollum 2004). A polymer based on poly(carbonate-urea)urethane and polyhedral oligomeric silsesquioxane nanoparticles, have been prepared and have reported the nanocomposite's heparin-like behaviour at the blood-material interface (Davis and Leach 2008). These efforts have indicated that although the composite polymers decrease thrombogenicity on their surfaces, still concerns over toxicity of carbon nanotubes remains (Kim, Khang et al. 2009). Nano-carbon materials are not biodegradable and will remain a permanent fixture in the area of vascular regeneration, thereby raising concerns regarding immunogenicity and toxicity.

2. Design for controlled degradation of scaffolds

Biodegradability is a must for polymer scaffolds on which culturing of cells is done to engineer new tissues or organs. Polyglycolic acid (PGA) is commonly used in tissue-engineering applications as it degrades through hydrolysis of its ester bonds, and glycolic acid, after that, is metabolized and eliminated as water and carbon dioxide (Langer and Tirrell 2004). PGA loses its strength in vivo within 4 weeks and is completely absorbed by 6 months (Ravi and Chaikof 2010).

Now to control the degradation rates of the scaffold, a composite can be prepared by incorporating other biodegradable polymers with PGA. This will allow us to sufficiently modify the time of degradation of the scaffold so as to give the required time necessary for tissue regeneration and proliferation. Things to consider while choosing such a material are:

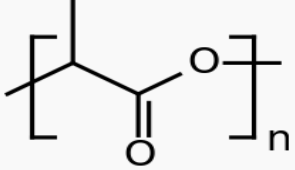
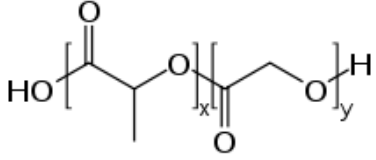
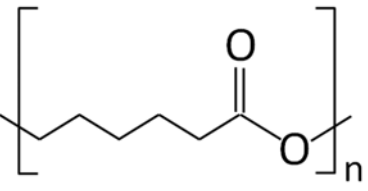
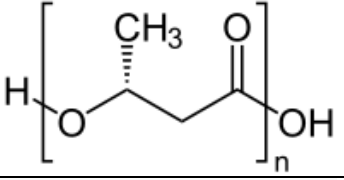
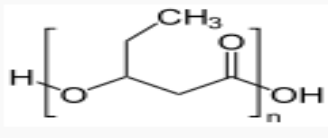
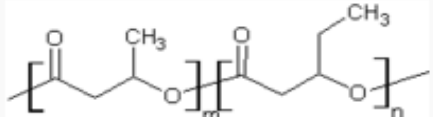
- Degradation time/rate of the material in vivo
- Degradation scheme of the material in vivo

Individual properties of each of these biodegradable polymers govern their use in design of composites for specific needs(Gunatillake, Meijs et al. 2001). Degradation rate and scheme of each of these polymers is different and a composite can be prepared by varying the ratio of these polymers which will give us:

- Predictable lot-to-lot uniformity
- Free from concerns of immunogenicity
- Reliable source of raw materials
- Tailor-able properties

A list of common degradable polymers which can be used are as follows(Gunatillake and Adhikari 2003):

Table 1: Chemical structures of commonly used degradable polymers

Polymer	Polymer repeat unit structure
Poly(lactic acid) PLA	
Poly(lactic-co-glycolic acid) PLGA	
Poly(caprolactone) PCL	
Poly-3-hydroxybutyrate P3HB	
poly-3-hydroxyvalerate PHV	
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV	

There are two main types of pathways observed when it comes to degradation of polymer composites(Gunatillake and Adhikari 2003). They are:

- Erosion from surface- Sample is eroded from surface, like PHBV, PHV, P3HB.
- Degradation in bulk– Whole sample gets degraded throughout, like PLA, PGA, PLGA, PCL.

Specific examples can be cited which will give us a better insight into the application of these special polymers in composites(Shum-Tim, Stock et al. 1999). Engineered an aortic graft consisting of a polymer scaffold of PGA and polyhydroxyoctanoate (PHO) seeded with bovine carotid artery cells. The inner layer of the construct was made of nonwoven mesh of PGA fibres, while the outer layers were composed of nonporous PHO. The PGA scaffold promoted cellular growth and ECM production, while the slower degradation rate of PHO provided mechanical support as this remodelling occurred. Significantly, the graft did not require extensive in vitro conditioning. The construct was implanted directly in the abdominal aorta of lambs with 100% patency noted at 5 months(Fu, Sodian et al. 2004).

Similarly another composite which was based on polycaprolactone (PCL) exhibits slow degradation by hydrolysis of ester linkages, and elimination of the resultant fragments by macrophages and giant cells(Shinoka, Shum-Tim et al. 1998). PCL-based scaffolds have been used to engineer venous blood vessels. The PCL–polylactic acid copolymer was reinforced with woven PGA and seeded with autologous smooth muscle and endothelial cells harvested from a peripheral vein. Subsequent studies have reported greater than 95% patency at a mean follow-up of 16 months(Watanabe, Shin'oka et al. 2001).

3. Design to manipulate mechanical behaviour

Developing the successful graft with adequate mechanical strength to withstand the pressure developed during the constant blood flow is really tough to achieve. Most of these biomaterials are mechanically weak, and unfortunately, these materials become even weaker when fabricated into porous scaffolds and/or used in vivo in physiological wet conditions significantly limiting their use in various tissue engineering applications(Konig, McAllister et al. 2009). Hence there is a dire need to improve the mechanical properties such as ultimate tensile strength, elastic modulus, compliance testing, suture retention strength, dynamic fatigue test and also burst pressure.

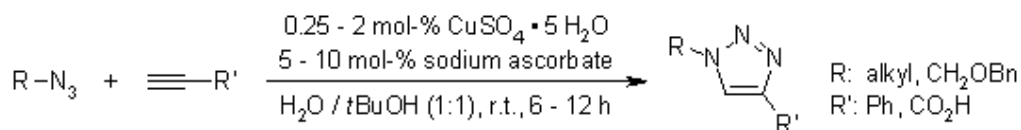
In terms of addressing concerns on the weak mechanical strength of the existing biopolymers, some traditional approaches can be employed to increase mechanical strength like:

- The introduction of urethane or amine groups into polyesters has been proved to be an effective way for improving mechanical strength of polyester elastomers.
- Increasing the cross-linking density may serve as another strategy.

However, improving mechanical properties by increasing polymer cross-linking densities and introducing urethane/urea bonds in elastomers sacrifice the limited functional groups for future bio conjugation, functionalizing and also unwanted adverse effects on the material degradation rate which limits their use in practical vascular tissue grafts.

A novel approach to obtain desirable mechanical properties can be sought in Click chemistry which plays a dual role in the design of biomaterials, greatly improving mechanical strength and providing easily clickable surfaces for biofunctionalization. This novel chemistry modification strategy is applicable to a number of different types of polymers(Díaz, Punna et al. 2004).

As one of the most effective, site-specific reactions that are tolerant to water, oxygen, and a wide range of functionalities, azide-alkyne cycloaddition (AAC, click chemistry) has been a promising method for functionalizing bio-related systems. It was also reported that the triazole rings resulting from click chemistry could imitate amide bonds serving as mechanical strength improving moieties(Guo, Xie et al. 2014). Herein, we have introduced usage of click chemistry into citrate-based biodegradable elastomers, CABEs to serve a dual role and create a novel material chemistry design strategy to simultaneously improve the bulk material mechanical strength and enable easy surface site-specific biofunctionalization, which can also be broadly applied to other functional biodegradable polymer design.

Equation 1: Reaction of the Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) (source: (Himo, Lovell et al. 2005) J. Am. Chem. Soc.)

Here we take example of citrate-based biodegradable elastomers (CABEs), and apply click chemistry to improve its mechanical properties and bio functionality. We use poly (1, 8-octanediol) citrate and introduce azide and alkyne functional diols, azide (pre-POC-N 3) and alkyne (pre-POC-AI) functionalized POC (poly (1, 8-octanediol citrate)) prepolymer monomers were synthesized. (Scheme 1 A). Pre-POC-N 3 and pre-POC-AI were mixed and cross-linked via a thermal synchronous binary (TSB) cross-linking mechanism. In the TSB cross-linking, thermal click reaction between azide and alkyne groups and esterification between $-\text{COOH}$ and $-\text{OH}$ groups (Hong, Luo et al. 2011), takes place simultaneously to form TSB cross-linked POC-click elastomers. POC-click elastomers possessed much improved mechanical strength (up to 40 MPa of tensile stress). The uniquely introduced extra azide groups on POC-click polymers also enabled the easy conjugation of heat-labile biomolecule such as peptides or proteins, which can effectively promote the adhesion and proliferation of endothelial cells (ECs), was exemplarily clicked onto POC-click films and scaffolds. (Johnson, Finn et al. 2008). Thus, the introduction of click chemistry into citrate-based biodegradable elastomer (CABE), such as poly (1, 8-octanediol citrate) (POC), can vastly increase the mechanical strength of the resulting polymer and greatly facilitate biomolecule conjugation through the clickable moieties (Li, Hill et al. 2005).

Thus appropriate polymers can be selected from the CABEs and then with the help of click chemistry, a suitable composite can be given which is appropriate in terms of the required tensile strength, young's modulus, elongation and degradation too.

4. Design for better Impact resistance

Impact resistance is the ability of a material to withstand a high force or shock applied to it over a short period of time. It is necessary for the graft to be impact resistant because in order to bear the shocks and stresses experienced in every-day activities. The aim of this work was to apply the helicoidal design strategy observed in the stomatopod dactyl club (Full, Caldwell et al. 1989) to the fabrication of high performance biomaterial composites (Patek and Caldwell 2005).

Five sets of composite panels, of equal dimensions are to be fabricated with fixed prepreg (pre-impregnated) layers that were laid up with different ply orientations. The first were unidirectional samples, with all plies oriented in the 0° direction. The second were quasi-isotropic panels consisting of prepreg layers oriented in the 0° , $\pm 45^\circ$ and 90° directions, with the layup symmetric about the mid-plane. For all helicoidal samples, prepreg plies were cut to specific angles prior to layup. All samples were fabricated to be symmetric about the mid-plane, meaning each right-handed rotation was immediately followed by a left-handed rotation. Mid-plane symmetry is critical in composite manufacturing, to prevent the warping that can occur in non-symmetric layups because of residual stresses that develop during elevated temperature cure. In addition to the unidirectional and quasi-isotropic controls, helicoidal structures can also be fabricated with rotation angles of 7.8° , 16.3° and 25.7° . These rotation angles were chosen such that all panels had a consistent thickness, and mid-plane symmetry could be maintained. Details of the composite layup schemes are presented in the following table. Stacking of these layers is stiffened by changes in temperature upto a certain point that makes the layers to comfortably settle on one another (Grunenfelder, Suksangpanya et al. 2014).

The composites formed due to this type of stacking gives helicoidal internal structure which when subjected to various impact tests, exhibits much better resistance when compared to a non-helicoidal structured composite.

FUTURE PROSPECTS

According to updated statistics, cardiovascular disease (CVD) has become a ubiquitous cause of morbidity and a leading contributor to mortality in most countries. Cardiovascular disorders represent the foremost cause of preventable death globally. With obesity, type II diabetes, hypertension, and other cardiovascular risk factors on the rise in developed countries, vascular systems engineering is gaining a more prominent position in the practice of preventative and restorative medicine (Go, Mozaffarian et al. 2013).

Table 2: Leading Causes of Death in 2011 (source: Centre for Disease Control and Prevention, USA)

Disease	Number of deaths in US
Heart disease	596,339
Cancer	575,313
Chronic lower respiratory diseases	143,382
Stroke(cerebrovascular diseases)	128,931
Alzheimer's disease	84,691

The limited availability of healthy autologous vessels for bypass grafting procedures has led to the fabrication of prostheticvascular conduits. Research in recent years has focused on tissue engineered heart valves (TEHV) and tissue engineered blood vessel (TEBV) substitutes as potential interventional treatments for specific cardiovascular disease pathologies. While synthetic polymers have been extensively studied as substitutes in vascular tissue engineering, they sometimes fail to meet the biological challenges at the material-blood interface. Various tissue engineering techniques have emerged to address these flaws and increase long-term patency of vascular grafts (Isenberg, Williams et al. 2006).

The development of a synthetic arterial substitute represents a major milestone of 20th century medicine, providing technology that has helped in saving lives of millions of patients. The challenges of creating the ideal tissue-engineered vascular substitute are plentiful, but meaningful progress has been made towards understanding the importance of both the physical and chemical parameters which govern the biologic requirements of biomaterials for vascular tissue engineering. Investigators continue to strive for the generation of multifunctional materials with optimized release and presentation of bioactive molecules in order to guide *in situ* vascular regeneration (Baguneid, Seifalian et al. 2006).

But still a major drawback of vascular tissue engineering that needs to be resolved is the time taken for these composites to make as well as the time taken for the fabrication of the entire implantable scaffold which ranges in weeks. Patients who come for vascular tissue grafts might not have this much time on their hands. Thus, a speedy way to develop TEBVs need to be proposed (Maas and Böger 2003).

CONCLUSION

Composites have gained prevalence in the field of tissue engineering due to the lack of individual biomaterials satisfying the multifunctional needs of regenerating tissue. In this review, we have appreciated how chemistry plays a vital role in determining the properties of the composite. At present, it is clear that some properties of biomaterial composites can be controlled by careful designing, such as thrombogenic potential, degradation rates, mechanical behaviour, impact resistance, size, shape, etc. which greatly impacts the functionality of a biomaterial in a biological environment after it gets placed. Although considerable advances have already been made in understanding how the physical and chemical properties of materials affect biological functions, the field is still in its infancy. Many other parameters including density and porosity will also significantly impact a material's function, but they need to be studied more carefully. This greatly widens the design parameter space for the next generation of biomaterials but simultaneously raises important queries. Considerable work remains to map the dependence of biological response to physical and chemical properties and to categorize the relative importance of each factor individually and may have to be elucidated case by case. Through continued collaboration among chemical and biomedical engineers, vascular surgeons, biologists, physicists and material scientists existing barriers in the creation of and vascular tissue substitutes will undoubtedly be broken.

REFERENCES

- Baguneid, M., A. Seifalian, H. Salacinski, D. Murray, G. Hamilton and M. Walker (2006). "Tissue engineering of blood vessels." *British Journal of Surgery* 93(3): 282-290.
- Barker, T. H. and S. C. Heilshorn (2014). "Biological Biomaterials Structure, Function, Property, Design across the Molecular-Nano-Micro-Macro Scales." *Acta biomaterialia*.
- Davis, H. and J. Leach (2008). "Hybrid and composite biomaterials in tissue engineering." *Topics in multifunctional biomaterials and devices* 1.
- Devine, C. and C. McCollum (2004). "Heparin-bonded Dacron or polytetrafluorethylene for femoropopliteal bypass: five-year results of a prospective randomized multicenter clinical trial." *Journal of vascular surgery* 40(5): 924-931.

- Díaz, D. D., S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin and M. Finn (2004). "Click chemistry in materials synthesis. 1. Adhesive polymers from copper-catalyzed azide-alkyne cycloaddition." *Journal of Polymer Science Part A: Polymer Chemistry*42(17): 4392-4403.
- Fu, P., R. Sodian, C. Lüders, T. Lemke, L. Kraemer, M. Hübner, Y. Weng, S. P. Hoerstrup, R. Meyer and R. Hetzer (2004). "Effects of Basic Fibroblast Growth Factor and Transforming Growth Factor-[beta] on Maturation of Human Pediatric Aortic Cell Culture for Tissue Engineering of Cardiovascular Structures." *ASAIO journal*50(1): 9-14.
- Full, R. J., R. L. Caldwell and S. W. Chow (1989). "Smashing energetics: prey selection and feeding efficiency of the stomatopod, *Gonodactylus bredini*." *Ethology*81(2): 134-147.
- Go, A. S., D. Mozaffarian, V. L. Roger, E. J. Benjamin, J. D. Berry, W. B. Borden, D. M. Bravata, S. Dai, E. S. Ford and C. S. Fox (2013). "Heart disease and stroke statistics--2013 update: a report from the American Heart Association." *Circulation*127(1): e6.
- Grunenfelder, L., N. Suksangpanya, C. Salinas, G. Milliron, N. Yaraghi, S. Herrera, K. Evans-Lutterodt, S. Nutt, P. Zavattieri and D. Kisailus (2014). "Bio-inspired impact-resistant composites." *Acta biomaterialia*.
- Gunatillake, P. A. and R. Adhikari (2003). "Biodegradable synthetic polymers for tissue engineering." *Eur Cell Mater*5(1): 1-16.
- Gunatillake, P. A., G. F. Meijs and S. J. McCarthy (2001). "Developments in design and synthesis of biostable polyurethanes." *Biomedical Applications of Polyurethanes* Eds Vermette, Griesser, Laroche, Guidoin, Landes Bioscience: 160-170.
- Guo, J., Z. Xie, R. T. Tran, D. Xie, D. Jin, X. Bai and J. Yang (2014). "Click chemistry plays a dual role in biodegradable polymer design." *Advanced Materials*26(12): 1906-1911.
- Himo, F., T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin (2005). "Copper (I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates." *Journal of the American Chemical Society*127(1): 210-216.
- Hong, J., Q. Luo, X. Wan, Z. S. Petrović and B. K. Shah (2011). "Biopolymers from vegetable oils via catalyst-and solvent-free "click" chemistry: Effects of cross-linking density." *Biomacromolecules*13(1): 261-266.
- Iozzo, R. V. (2001). *Proteoglycan protocols*, Springer.
- Isenberg, B. C., C. Williams and R. T. Tranquillo (2006). "Small-diameter artificial arteries engineered in vitro." *Circulation research*98(1): 25-35.
- Jenkins, A. D., P. Kratochvil, R. F. T. Stepto and U. W. Suter (1996). *Glossary of basic terms in polymer science (IUPAC Recommendations 1996)*. Pure and Applied Chemistry. 68: 2287.
- Johnson, J. A., M. Finn, J. T. Koberstein and N. J. Turro (2008). "Construction of Linear Polymers, Dendrimers, Networks, and Other Polymeric Architectures by Copper-Catalyzed Azide-Alkyne Cycloaddition "Click" Chemistry." *Macromolecular Rapid Communications*29(12-13): 1052-1072.
- Kannan, R. Y., H. J. Salacinski, P. E. Butler, G. Hamilton and A. M. Seifalian (2005). "Current status of prosthetic bypass grafts: a review." *Journal of Biomedical Materials Research Part B: Applied Biomaterials*74(1): 570-581.
- Kim, J. Y., D. Khang, J. E. Lee and T. J. Webster (2009). "Decreased macrophage density on carbon nanotube patterns on polycarbonate urethane." *Journal of Biomedical Materials Research Part A*88(2): 419-426.
- Konig, G., T. N. McAllister, N. Dusserre, S. A. Garrido, C. Iyican, A. Marini, A. Fiorillo, H. Avila, W. Wystrychowski and K. Zagalski (2009). "Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery." *Biomaterials*30(8): 1542-1550.
- Langer, R. and D. A. Tirrell (2004). "Designing materials for biology and medicine." *Nature*428(6982): 487-492.
- Li, C., A. Hill and M. Imran (2005). "In vitro and in vivo studies of ePTFE vascular grafts treated with P15 peptide." *Journal of Biomaterials Science, Polymer Edition*16(7): 875-891.
- Maas, R. and R. H. Böger (2003). "Old and new cardiovascular risk factors: from unresolved issues to new opportunities." *Atherosclerosis Supplements*4(4): 5-17.
- Patek, S. and R. Caldwell (2005). "Extreme impact and cavitation forces of a biological hammer: strike forces of the peacock mantis shrimp *Odontodactylus scyllarus*." *Journal of Experimental Biology*208(19): 3655-3664.
- Ravi, S. and E. L. Chaikof (2010). "Biomaterials for vascular tissue engineering." *Regenerative medicine*5(1): 107-120.
- Sakiyama-Elbert, S. E. (2014). "Incorporation of heparin into biomaterials." *Acta biomaterialia*10(4): 1581-1587.
- Shinoka, T., D. Shum-Tim, P. X. Ma, R. E. Tanel, N. Isogai, R. Langer, J. P. Vacanti and J. E. Mayer Jr (1998). "Creation of viable pulmonary artery autografts through tissue engineering." *The Journal of thoracic and cardiovascular surgery*115(3): 536-546.

- Shum-Tim, D., U. Stock, J. Hrkach, T. Shinoka, J. Lien, M. A. Moses, A. Stamp, G. Taylor, A. M. Moran and W. Landis (1999). "Tissue engineering of autologous aorta using a new biodegradable polymer." *The Annals of thoracic surgery*68(6): 2298-2304.
- Watanabe, M., T. Shin'oka, S. Tohyama, N. Hibino, T. Konuma, G. Matsumura, Y. Kosaka, T. Ishida, Y. Imai and M. Yamakawa (2001). "Tissue-engineered vascular autograft: inferior vena cava replacement in a dog model." *Tissue engineering*7(4): 429-439.
- Weinberg, C. B. and E. Bell (1986). "A blood vessel model constructed from collagen and cultured vascular cells." *Science*231(4736): 397-400.